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CLINICAL PROTOCOL

A randomized open label trial of spironolactone versus prednisolone in corticosteroid-naïve boys with DMD

EXEMPT IND #: 136166

PROTOCOL VERSION: 7.0 (08Mar2021)

Sponsor-Principal Investigator: Kevin M. Flanigan, M.D., Nationwide Children's Hospital

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2. PROTOCOL SYNOPSIS

Name of Sponsor:	Kevin M. Flanigan, M.D.
Name of Investigational Product:	Spironolactone
Name of Active Ingredient:	Spironolactone
Study Title:	A randomized open label pilot trial of spironolactone versus prednisolone in corticosteroid-naïve boys with DMD
Study Number:	N=24
Sponsor-Principal Investigator:	Kevin M. Flanigan, M.D.
Clinical Study Phase:	Pilot Study
Number of Centers:	Four to seven centers
Study Duration:	6 months. Subjects will undergo strength and functional testing at screening (Day -14 to 0) and monthly electrolyte testing. Follow up strength and functional testing will occur on Days 90 and 180. After 6 months of treatment, all subjects will be offered standard glucocorticoid therapy based on their local clinic's standard of care or continued dosing on the medication taken during the trial if there is clear established efficacy.
Study Design:	Randomized, open-label, pilot clinical trial of spironolactone suspension versus oral prednisolone
Study Objectives:	To determine if 6 months of treatment with either spironolactone or a standard clinical dose of corticosteroids results in equivalent improvement in time to complete the 100 meter timed test (100M). To assess the safety of spironolactone treatment in the steroid naïve DMD population.
Patient Population:	**Inclusion Criteria:** • Duchenne muscular dystrophy (DMD) patients ≥4 to ≤7 years of age • Clinical features of DMD that include proximal predominant weakness and/or gait disturbance • Presence of a truncating mutation of the *DMD* gene in the patient or an affected male relative OR a muscle biopsy that demonstrates <5% dystrophin in the patient or an affected male relative • Normal left ventricular ejection fraction by screening echocardiogram • Ability to cooperate for testing

	• No prior treatment with glucocorticoids or vamorolone • No concomitant experimental therapies
	Exclusion Criteria: • Subject amenable to or currently being treated with eteplirsen, golodirsen, casimersen, or viltolarsen • Hyperkalemia at screening • History of or ongoing renal failure (elevated creatinine, oliguria, anuria) • Hypersensitivity to spironolactone (rash, respiratory distress, arrhythmia, numbness or tingling of extremities) • Current treatment with an ACEi • Severe peptic ulcer disease or recent gastrointestinal perforations
Study Procedures:	Subjects will receive either prednisolone or spironolactone. Strength and functional testing will occur at screening (Day -14 to 0) and at 90 and 180 days after initiation.
Study Outcomes:	**Primary:** • Time to complete the 100 meter timed test (100M)
	Secondary: • Muscle strength as measured by dynamometry score, a composite score made up of values from maximum voluntary isometric contraction testing of the flexors and extensors of the elbows and knees, as measured in kilograms of force. • North Star Ambulatory Assessment (NSAA), a functional scale validated in the DMD population • 4-stair climb • Time to arise from floor
	Exploratory: • Safety measures • Biomarker studies
Sample Size:	A total of 24 subjects are expected to be enrolled in this trial, with 12 randomized to each treatment group. • **Treatment Group 1:** Spironolactone 1 mg/kg/day (n=12 subjects) • **Treatment Group 2:** Prednisolone 0.75 mg/kg/day (n=12 subjects) or weekend dosing per the sites standard of care

Statistical Analysis:	Age-corrected 100M will be considered as the primary outcome, and will be evaluated using a two-one-sided t-test to test equivalency between treatment arms in the change from baseline to 6 months. In addition, repeated measures ANOVA will be used to determine whether the trajectory across all three time points (baseline, 3mo, 6mo) differs by treatment group. Strength testing/dynamometry, NSAA, 4-stair climb, and time to arise from floor scores will be secondary outcomes, and will also be compared by treatment group using repeated measures ANOVA. Biomarkers studies will be considered exploratory outcomes. Power: Prior studies evaluating the 100m walk test among patients with DMD have shown an average change over 6 months of -2.9 ±7.8. An effective sample size of 10 patients per group will provide 80% power to declare equivalency with an equivalence margin of ±10.6.
Long-term follow-up:	All adverse events will be followed until resolution or stabilization.

3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Table 1 Abbreviations and Specialist Terms

Abbreviation	Definition
ACEi	Angiotensin Converting Enzyme Inhibitor
DMD	Duchenne muscular dystrophy
MDA	Muscular Dystrophy Association
MR	Mineralocorticoid Receptor
NSAA	North Start Ambulatory Assessment
Sponsor-Investigator	Nationwide Children's Hospital Principal Investigator
Principal Investigator	Site Principal Investigators

4. KEY ROLES

This clinical trial is a randomized, open-label, pilot study in which either spironolactone or prednisolone suspension will be delivered daily by mouth in steroid naïve DMD subjects.

Kevin Flanigan, M.D. will serve as the Sponsor and Principal Investigator (Sponsor-Investigator) for this trial. Dr. Flanigan received clinical fellowship training in Neuromuscular Disorders at the Johns Hopkins University, and post-doctoral training in Human Molecular Biology and Genetics at the University of Utah. He is co-Director of the Nationwide Children's Hospital Muscular Dystrophy Association (MDA) Clinic, and has extensive experience in translational research in DMD and related myopathic disorders. He has participated as an investigator in multiple clinical trials in the muscular dystrophies, including trials of nonsense suppression,[1, 2] exon skipping,[3, 4] and myostatin inhibition[5]. He currently holds one active gene therapy IND: Phase I gene transfer clinical trial for Duchenne Muscular Dystrophy using rAAVrh74.MCK.GALGT2 (IND 16175). He is the principal investigator and original IND initiating investigator of two ongoing trials: Phase I/II gene transfer clinical trial of scAAV9.U1a.SGSH for treatment of mucopolysaccharidosis type IIIA (Sanfilippo Syndrome type A) (IND 16850) and rAAV9.CMV.NAGLU for the treatment of mucopolysaccharidosis type IIIB (IND 16671).

Megan Waldrop, M.D., an Assistant Professor at Nationwide Children's Hospital/Ohio State University will serve as the lead Co-Investigator for this trial. Dr. Waldrop is board-certified in Neurology with special qualifications in Pediatric Neurology and Neuromuscular Medicine. She is currently a co-investigator on six active gene therapy trials at NCH, including a phase I gene transfer clinical trial for Duchenne Muscular Dystrophy using rAAVrh74.MCK.GALGT2 (IND 16175), a phase I/II trial of rAAV9.CMV.NAGLU for the treatment of mucopolysaccharidosis type IIIB (Sanfilippo Syndrome type B) (IND 16671), a phase I/II gene transfer clinical trial of scAAV9.U1a.SGSH for the treatment of mucopolysaccharidosis type IIIA (Sanfilippo Syndrome type A) (IND 16850), and three clinical trials of AVXS-101 for the treatment of Spinal Muscular Atrophy (IND 15699).

Jill Rafael-Fortney, PhD, will serve as Co-Investigator for this trial and may conduct the biomarker studies using the samples collected as part of this trial. Dr. Rafael-Fortney has a B.A. from Cornell University in Genetics/Biology, a Ph.D. in Human Genetics from University of Michigan, and completed a postdoctoral fellowship at the University of Oxford. She is a Professor of Physiology & Cell Biology at The Ohio State University and has vast expertise, starting during her Ph.D. studies, in the genetic, histological, cell biological, and biochemical characterization of skeletal and cardiac muscle pathologies related to Duchenne muscular dystrophy (DMD). Her graduate work led to the dissection of the molecular structure-function relationships of the dystrophin protein that was used as a basis for dystrophin-based gene therapy

clinical trials and she developed the utrophin/dystrophin-deficient (dko) mouse model of DMD during her postdoctoral fellowship. Most recently, her lab has carried out numerous preclinical and mechanistic studies demonstrating efficacy of mineralocorticoid receptor antagonists for cardiac and skeletal muscle pathologies in DMD mouse models. She was part of the team that has published the translation of mineralocorticoid receptor antagonist efficacy for DMD cardiomyopathy (ClinicalTrials.gov identifier NCT01521546).

Linda Cripe, M.D., a Professor of Pediatrics at Nationwide Children's Hospital/The Ohio State University will serve as a Co-Investigator for this trial. Dr. Cripe is a board-certified Pediatric Cardiologist with sub-specialty interest in the evaluation and treatment of the cardiomyopathy associated with neuromuscular disorders, specifically Duchenne muscular dystrophy. She received a B.S. in biochemistry from The University of Iowa and a M.D. from The University of Iowa College of Medicine. She completed a pediatric residency at the University of Iowa Hospitals and Clinics and a pediatric cardiology fellowship at The University of Iowa Hospitals and Clinics and Boston Children's Hospital/Harvard Medical School. Her work has focused on defining the natural history of the DMD associated cardiomyopathy by non-invasive imaging specifically by echocardiography and cardiac magnetic resonance imaging.

4.1 Clinical Trial and Principal Investigator

The trial will be carried out at the Abigail Wexner Research Institute at Nationwide Children's Hospital. Dr. Kevin Flanigan, Professor of Pediatrics and Neurology at the Ohio State University, and Center Director of the Center for Gene Therapy at the Abigail Wexner Research Institute at Nationwide Children's Hospital, and Co-Director of the Muscular Dystrophy Clinic, will serve as the Sponsor-Principal Investigator. All Co-Investigators and site investigators have extensive experience in the performance of clinical trials in the neuromuscular pediatric population.

5. INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

5.1 Background

We propose a randomized open label pilot clinical trial of spironolactone versus prednisolone in corticosteroid-naïve boys ages 4-7 years old. The glucocorticoids prednisolone and deflazacort have been shown to improve muscle strength and to prolong ambulation in boys with DMD[8]. The effects in mdx mice are controversial and neither has clearly been shown to reverse skeletal muscle pathology in human disease or in animal models. The mechanism of action of corticosteroids is unclear, but several have been suggested, including stabilization of muscle membranes, diminishing the activation of calcium dependent proteases (and thus a decrease in subsequent myofiber degeneration), and decreasing intramuscular fibrosis. Corticosteroids have a significant side effect profile that includes obesity, cushingoid features, osteoporosis, and

behavioral disturbances. These side effects become pronounced after what may be a decade or more of treatment in boys with DMD, and often are the cause of cessation of therapy or subtherapeutic dosing in the shorter term.

Spironolactone is an aldosterone antagonist primarily used as a potassium sparing diuretic. However, it also has anti-androgen effects, and is used to treat a variety of conditions, including hirsutism in women. Its side effect profile is well known, and includes gynecomastia and hyperkalemia. It is widely used, however, in the pediatric population, with limited side effects[9]. A recent study by Dr. Rafael-Fortney, a Co-Investigator, evaluated the effect of spironolactone treatment in the mdx mouse (the standard animal model of DMD) and mdx:utr+/- mouse also lacking 1 copy of utrophin since these mice demonstrate more severe histopathologic changes (including markedly increased fibrosis)[10]. In several published studies, a combined treatment with the angiotensin converting enzyme (ACE) inhibitor lisinopril and mineralocorticoid receptor (MR) antagonist spironolactone reduces ongoing skeletal muscle damage and improves muscle function in DMD mouse models[10-12]. These include improvements of cardiac, limb, and diaphragm function approaching 80% of normal following early treatment (at 4 weeks after birth), an extraordinary effect in comparison to many other preclinical studies of compounds that have been tested in humans – besting, for example, the protection provided by prednisone in the mdx mouse[11]. These preclinical results have already been translated into a published double-blind, placebo controlled clinical trial with the MR antagonist eplerenone showing efficacy in primary cardiac outcomes in DMD patients[13]. Due to the inclusion criteria for this trial, many patients were already nonambulatory and skeletal muscle function was not assessed. A second non-inferiority double-blind trial comparing specific and non-specific MR antagonists with primary cardiac outcomes is currently underway.

Spironolactone and lisinopril target the mineralocorticoid receptor directly and indirectly, respectively, through the renin-angiotensin-aldosterone system. Treatment with lisinopril alone improves histopathology, but cannot recapitulate improvements in skeletal muscle function of dystrophic mice after combined treatment with spironolactone[14]. Dr. Rafael-Fortney has recently shown that the MR is present in both mouse and human skeletal muscle fibers[15]. Treatment of normal human myotubes with aldosterone results in a large number of gene expression changes, supporting MR functions as a nuclear hormone receptor in skeletal muscles[15]. Treatment of dystrophic mice also demonstrates increased muscle membrane stabilization as demonstrated by laser injury assays[16].

We have now also shown that infiltrating myeloid cells clustered in damaged areas of dystrophic mouse skeletal muscles have the capacity to produce the natural ligand of the MR, aldosterone[16], which in excess is known to exacerbate tissue damage. Aldosterone synthase protein levels are increased in leukocytes isolated from dystrophic mouse muscles compared with controls and local aldosterone levels in dystrophic skeletal muscles are increased, despite normal circulating

levels. 11β-HSD2, the enzyme that inactivates glucocorticoids to increase MR selectivity for aldosterone, is also increased in dystrophic mouse muscle tissues[16]. These data suggest MR activation is in excess of physiological need, likely contributes to the pathology of muscular dystrophy, and explains the likely mechanism of preclinical efficacy of MR antagonists in skeletal muscles and heart. Since DMD patients have even more muscle inflammation than dystrophic mice, this approach has the potential to be even more efficacious in patients.

The pilot trial we propose will directly answer the question of whether spironolactone is as efficacious as prednisolone over 6 months of treatment in young patients (age ≥4 to ≤7 years) with DMD, as judged by the age-corrected 100 meter timed test and by dynamometry scores derived maximum voluntary isometric contraction testing of selected muscles and the time to climb 4 stairs. For steroid-naïve patients, this could delay institution of prednisolone therapy – considered the standard of care – for up to 6 months. However, it is important to recognize that not all DMD parents choose for their boys to go on steroids during this age window, and that there is no clear evidence based consensus of when treatment must be initiated. Finally, we note that published data from Dr. Rafael-Fortney's lab and others demonstrates that prednisolone results in increased cardiac deterioration in the mdx mice, suggesting cardio-toxic effects on dystrophic heart[11, 17]. Therefore, we feel that delaying onset of steroid therapy is justified for this pilot trial in order to gain meaningful pilot data regarding spironolactone in DMD patients, and note that all subjects will be offered open label prednisolone therapy as a standard of care after completion of the trial. If there is equal or greater efficacy with spironolactone, they may choose to continue on spironolactone.

In this pilot trial we will use prednisolone since it is delivered as an oral suspension. Since the standard of care regimen (daily versus weekend dosing of prednisolone) differs from site to site, each PI will have the discretion to assign subjects to their preferred regimen. Equal efficacy has been shown between daily and weekend dosing regimens[18].

5.2 Rationale

DMD is a devastating disorder of childhood, leading to loss of ambulation by age 12 years, and typically to death between 20 and 30 years. Until recently, the only treatment shown to improve strength and preserve ambulation in DMD patients was the use of glucocorticoids, which are accompanied by significant side effects. Recent developments in gene corrective therapies (such as the exon-skipping oligomer eteplirsen or the nonsense-suppression compound ataluren) represent potential therapies only for those relatively small subsets of patients with mutations that are amenable to that specific corrective mechanism (around 13% and 15% of DMD patients, respectively)[19]. We wish to determine whether another commonly used medication, spironolactone, may have similar beneficial effects with a lower side effect profile and be applicable to a wider population of DMD patients.

Our hypothesis for this controlled pilot trial is that spironolactone and prednisolone are of equal efficacy in improving skeletal muscle function over a 6-month period, and that spironolactone will be well tolerated in this patient population.

One outcome is that both drugs demonstrate equal efficacy in motor function. This would then serve as pilot data for a longer term study.

6. OBJECTIVES AND PURPOSE

6.1 Primary Outcome

The primary objective of this trial is to determine whether spironolactone has similar efficacy to glucocorticoids in improving muscle strength in steroid naïve DMD patients. Glucocorticoids are the only medications that have been shown to change the clinical course of Duchenne muscular dystrophy, but have significant side effects (e.g., obesity, cushingoid features, osteoporosis, and behavioral disturbances). Preclinical data suggests a significant protective effect from spironolactone in the mdx and mdx;utrn+/- animal models. We propose a randomized, open-label pilot trial comparing spironolactone to prednisolone.

The primary outcome measure will be the time to complete a 100 meter timed test (100M)[20].

6.2 Secondary Outcome

Secondary outcome measures will be Dynamometry score, which is a summation of maximum voluntary isometric contraction test values for knee flexion, knee extension, elbow flexion, and elbow extension; 4-stair climb; North Star Ambulatory Assessment (NSAA); and time to arise from the floor.

6.3 Exploratory Outcome

An exploratory outcome is to assess the safety of delivering spironolactone to the steroid naïve DMD population. Safety data will be monitored on a continual basis throughout the trial by the Principal Investigator and study staff.

Additional exploratory outcomes include biomarker studies.

7. STUDY DESIGN AND ENDPOINTS

7.1 Overall Study Design

The proposed trial will involve 24 subjects with DMD. Because DMD is an X-linked disorder, all subjects will be boys. Subject selection will not exclude anyone on the basis of race or ethnic background.

12 subjects will be randomized to each treatment group as follows:

- **Treatment Group 1:** Spironolactone 1 mg/kg/day (n=12 subjects)
- **Treatment Group 2:** Prednisolone 0.75 mg/kg/day (n=12 subjects) or weekend dosing as per sites standard of care

7.2 Study Population

DMD patients (n =24) will be enrolled in a randomized open label controlled trial of prednisolone (n= 10) versus spironolactone (n = 10) based on inclusion criteria as defined below. Four to seven collaborating centers will participate. Up to 24 subjects (~ 3 at each site) can be enrolled to include a 10% drop-out rate.

Power: Prior studies evaluating the 100m walk test among patients with DMD have shown an average change over 6 months of -2.9 ±7.8. An effective sample size of 10 patients per group will provide 80% power to declare equivalency with an equivalence margin of ±10.6[21].

Siblings will be permitted to participate. When sibling participation arises, the randomization will proceed as if they were unrelated.

7.3 Number of Subjects

A total of 24 subjects will be enrolled.

7.4 Dosing Plan

This is a randomized open label controlled pilot trial comparing spironolactone suspension and prednisolone. Medications will be provided by each institutional pharmacy. Each subject will be provided with a 90-day supply of medication. Subjects will be enrolled for a period of 6 months. Specific criteria for administering the drugs are as follows:

Patients fulfilling entry criteria will be enrolled in the trial and receive medication at designated dosing. They will receive either prednisolone 0.75 mg/kg/day, or spironolactone 1 mg/kg/day. Weekend prednisolone dosing as per sites standard of care is an acceptable alternative.

Subjects will be weighed at screening, Month 3, and Month 6. Weight based dose adjustments to the study drug should be made by the Study Doctor.

7.5 Spironolactone Suspension

Carospir® (spironolactone) 25 mg/5ml oral suspension is commercially available as a white to off-white, opaque, banana-flavored suspension and can be stored at room temperature. The product is manufactured by CMP Pharma, Inc. Study drug will be dosed daily at 1mg/kg.

Although spironolactone is commercially available in a suspension, it is NOT therapeutically equivalent to the tablets. The suspension results in 15% to 37% higher serum concentration compared to the tablet. Pediatric dosing is based on experience with tablets and extemporaneously compounded suspension.

7.6 Prednisolone

The Study Doctor at each study site will prescribe their preferred regimen of prednisolone. Daily dosing at 0.75 mg/kg/day or weekend dosing as per sites standard of care is acceptable. Study drug will be provided by the pharmacy at each study site every 90 days. The product is manufactured by Morton Grove Pharmaceuticals. If at any point the drug becomes unavailable from this manufacturer, another manufacturer can be used to obtain the drug until available from Morton Grove Pharmaceuticals.

Prednisolone is available commercially as a 15mg/5ml solution. It can be stored at room temperature. It is a clear solution with a grape odor and flavor. It contains 1.8% alcohol.

7.7 Establish Subject Identification Number

Research subjects will be assigned a randomization number (i.e., 1001, 1002, sequentially) prior to enrollment to facilitate randomization to one of two treatment groups, Spironolactone Treatment Group or Prednisolone Treatment Group. Upon consent, every research subject will be assigned a Subject Identification code. This will be done prior to any research procedures being scheduled, as they will require a Subject ID.

The Subject Identification code is generated as follows:

1) 3 letter site identifier
2) 2 digit study number
3) 3 digit sequential number of all candidates being screened

Example: Subject ID: NCH-01-001

8. STUDY ENROLLMENT AND WITHDRAWAL

24 male DMD subjects, ages ≥4 to ≤7 years who are steroid naïve will be enrolled in this pilot clinical trial. Subjects will be of any racial or ethnic background. Enrollment will be ongoing. The assessment and full treatment plan will be used for all subjects.

8.1 Subject Inclusion Criteria

Subjects must meet all of the following Inclusion Criteria:

1. Duchenne muscular dystrophy (DMD) patients ≥4 to ≤7 years of age
2. Clinical features of DMD that include proximal predominant weakness and/or gait disturbance

3. Presence of a truncating mutation of the *DMD* gene in the patient or an affected male relative OR a muscle biopsy that demonstrates <5% dystrophin in the patient or an affected male relative
4. Normal left ventricular ejection fraction by screening echocardiogram
5. Ability to cooperate for testing
6. No prior treatment with glucocorticoids or vamorolone
7. No concomitant experimental therapies

8.2 Subject Exclusion Criteria

Subjects must not meet any of the following Exclusion Criteria:

1. Subject amenable to or currently being treated with eteplirsen, golodirsen, casimersen, or viltolarsen
2. Hyperkalemia at screening
3. History of or ongoing renal failure (elevated creatinine, oliguria, anuria)
4. Hypersensitivity to spironolactone (rash, respiratory distress, arrhythmia, numbness or tingling of extremities)
5. Current treatment with an ACEi
6. Severe peptic ulcer disease or recent gastrointestinal perforations

8.3 Randomization

Nationwide Children's Hospital pharmacy will serve as the lead pharmacy. Randomization will be determined via the lead pharmacy. Subjects at each site will be randomized 1:1 to receive treatment with either prednisolone at 0.75 mg/kg/day (or weekend dosing per sites standard of care) or spironolactone at 1mg/kg/day. Subjects will be assigned treatment sequentially from the provided list. Subjects will not be randomized until the echocardiogram and all lab results are finalized and reviewed by the Study Doctor.

8.4 Baseline Assessment of Endpoints

After obtaining informed consent and completing the registration procedures, baseline subject history data will be collected, including records of all medications and supplements that the subject is taking. The following assessments will be performed to confirm subject eligibility for this trial. Screening tests must be completed prior to treatment administration as described in the study timeline. Screening testing includes the following, which are discussed in detail below:

- 100 meter timed test (100M)
- Strength testing and dynamometry
- North Star Ambulatory Assessment (NSAA)
- 4-stair climb
- Time to arise from floor
- Echocardiogram
- Baseline laboratory testing

The subject and his parents will be aware that his participation may be terminated after any of these screening procedures.

There is no central laboratory for this trial. Site-specific normal lab ranges will be documented in respective sites' regulatory binders. Lab ranges will be provided by each site's laboratory. Laboratory tests with values within the clinically significant range will be repeated during the same visit whenever possible. If the test result returns after the subject leaves the clinic, they will be immediately contacted. Subjects will be asked to return to the outpatient clinic for a repeat test. For non-local subjects, arrangements can be made to have the blood test redrawn in a laboratory close to home or by their primary care physician. If still out of the range and clinically significant, the subject may be terminated from the study following discussion with the Clinical Safety Monitor.

To avoid any confusion for the primary care physician, he or she will be informed (with permission from the subject) of their participation in the trial at the time of enrollment.

8.5 Subject Withdrawal Criteria

Subjects meeting the following criteria will be withdrawn from the trial:

1. Unable to tolerate study medication.
2. Failure to keep an accurate medication log.
3. Failure to keep study appointments.
4. Missing more than 10% of prescribed doses of study medication.*

**If a subject is missing more than 10% of prescribed doses, a discussion about the subject's continuation in the trial will take place between the Principal Investigator and the Sponsor-Investigator.*

Corticosteroids are the standard of care for the treatment of Duchenne muscular dystrophy and have a well-known side effect profile (see section 9.3). Subjects with any Grade 1 or higher (according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 and listed in section 11.1) adverse events that are anticipated and related to prednisolone use, will continue in the trial. Subjects with any Grade 2 or higher adverse events that are anticipated and are possibly, probably or definitely related to spironolactone use will have the study drug held and will be re-challenged in 1 week. If a subject experiences a recurrence of the adverse event, he may be withdrawn after discussion with the Clinical Safety Monitor. Subjects with any Grade 2 or higher adverse events that are unanticipated, may be withdrawn after discussion with the Clinical Safety Monitor. The Principal Investigator will determine a clinically appropriate tapering regimen with respect to assigned study drug and length of time on drug.

Principal Investigators must report all participant withdrawals to the Sponsor-Investigator within 48 hours of learning of the withdrawal. The Sponsor-Investigator will then notify the Clinical Safety Monitor of the withdrawal within 3 business days of the Sponsor-Investigator learning of the withdrawal.

9. STUDY AGENT

Site pharmacies will dispense spironolactone and prednisolone per the site's standard procedures. In the rare instance of a subject not being able to tolerate prednisolone, an equivalent dose of prednisone may be provided as crushed tablets after approval by the Sponsor-Investigator.

9.1 Dispensing of Study Agent

Subjects will be provided a supply of study drug at Day 0 and Month 3 (Day 90). A +/- 3 day window is acceptable for all visits after Day 0.

9.2 Description of Study Drug

Table 2 Investigational Product

Product Name	Spironolactone, USP
Dosage Form	25mg/5mL Oral Suspension
Route of Administration	Oral
Physical Description	Spironolactone, USP is practically insoluble in water, soluble in alcohol and freely soluble in benzene and in chloroform. Carospir Oral suspension contains 25 mg of the aldosterone antagonist spironolactone per 5 mL. Inactive ingredients include sorbic acid, potassium sorbate, citric acid anhydrous, sodium citrate dehydrate, simethicone emulsion, saccharin sodium, xantham gum, Magnasweet 110, glycerin, banana flavor, and purified water.
Manufacturer	CMP Pharma, Inc.

Product Name	Prednisolone Sodium Phosphate*, USP
Dosage Form	15 mg/5 mL Solution
Route of Administration	Oral

Physical Description	Prednisolone Sodium Phosphate Oral Solution equivalent to 15 mg prednisolone per 5 mL contains the following inactive ingredients: alcohol 1.8%, dibasic sodium phosphate, glycerin, liquid sugar, monobasic sodium phosphate, natural and artificial grape flavor, purified water, sodium benzoate and sorbitol solution. It may contain 10% dibasic sodium phosphate solution and/or 10% monobasic sodium phosphate solution to adjust pH between 6.0 and 8.0. Prednisolone sodium phosphate occurs as white or slightly yellow, friable granules or powder. It is freely soluble in water; soluble in methanol; slightly soluble in alcohol and in chloroform; and very slightly soluble in acetone and in dioxane.
Manufacturer	Morton Grove Pharmaceuticals**

Contact the NCH study team if substituting with prednisone tablets.

****If at any point the drug becomes unavailable from this manufacturer, another manufacturer can be used to obtain the drug until available from Morton Grove Pharmaceuticals.***

9.3 Potential Risks

Potential risks to study subjects include risks associated with administration of the study agent and with study procedures.

9.3.1 Risks Associated with Spironolactone

Common side effects associated with the use of spironolactone in this subject population may include: diarrhea, cramping, nausea, vomiting, somnolence, headache, ataxia, gynecomastia and urticaria.

9.3.2 Risks Associated with Corticosteroid Use

Common side effects associated with the use of prednisolone in this subject population may include: depression, euphoria, emotional instability, headache, seizures, vertigo, body fluid retention, hypernatremia, hypertension, acne, bruising, wound healing impairment, decreased body growth, increased risk of infection, diaphoresis, abdominal distention, pancreatitis, peptic ulcers, ulcerative esophagitis, hepatic impairment, steroid myopathy, and muscle weakness. Serious side effects may include: anaphylaxis, Cushing's syndrome, hyperglycemia, and primary adrenocortical insufficiency.

10. STUDY PROCEDURES AND SCHEDULE

Subjects will complete study assessments monthly via online survey, phone, or at clinic visits for 6 months.

10.1 Laboratory Procedures/Evaluations

Laboratory tests with values within the clinical significant range may be repeated during visits whenever possible. If the test result returns after the subject leaves the clinic and the Study Doctor wants additional tests, they will be immediately contacted. Subjects may be asked to return to the outpatient clinic for a repeat test. For non-local subjects, arrangements can be made to have the blood test redrawn in a laboratory close to home or by their primary care physician.

10.1.1 Clinical Laboratory Evaluations

Functional Testing

- 100 meter timed test (100M)
- Strength testing and Dynamometry
- North Star Ambulatory Assessment (NSAA)
- 4-stair climb
- Time to arise from floor

Blood Chemistry

Chemistry analysis will include the following at specified visits:

- Creatine Kinase (Visits 1, 8)
- Gamma Glutamyl Transferase (GGT) (Visits 1, 5, 8)
- Creatinine (Visits 1, 5, 8)
- Blood Urea Nitrogen (BUN) (Visits 1, 5, 8)
- Glucose (Visits 1, 5, 8)
- Cystatin C (Visits 1, 5, 8)
- Electrolytes (Sodium, Potassium, Chloride, CO_2) [All Visits]

Cardiac

Echocardiogram (Echo) will be conducted at the time points specified in Table 3.

Research Blood Sample

Blood samples will be collected for future research purposes, to examine/research potential disease modifiers and/or biomarkers. Research blood samples may be distributed for future research to Jill Rafael-Fortney, PhD, at Ohio State University, a Co-Investigator on this study.

10.2 Study Visits

10.2.1 Timeline

Table 3 Schedule of Assessments

Assessment	Visit 1	Study Drug Start[1]	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
	Screening		Week 1	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
	Day -14 to 0	Day 0	Day 7	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
Research Purposes Only									
Echocardiogram (Echo)[2]	X								X
Biomarkers Blood Draw (plasma and white blood cells)	X								X
Strength Testing/Dynamometry	X					X			X
Standard of Care									
Informed Consent	X								
AE Assessment		X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X
Physical Exam	X					X			X
Skin Exam	X					X			X
Cushingoid Exam	X					X			X
Medical History Review	X					X			X
100 Meter Timed Test	X					X			X
Parent Survey			X	X	X		X	X	
4-Stair Climb	X					X			X
North Star Ambulatory Assessment (NSAA)	X					X			X
Time to Arise from Floor	X					X			X
Creatine Kinase	X								X
Gamma Glutamyl Transferase (GGT)	X					X			X
Creatinine	X					X			X
Blood Urea Nitrogen (BUN)	X					X			X
Glucose	X					X			X
Cystatin C	X					X			X
Electrolytes[3]	X		X	X	X	X	X	X	X
Medication Dispensing[4]		X[1]				X			
Vitals	X					X			X

1. Study drug will be dispensed and treatment initiated once eligibility is confirmed following receipt and review of results from screening visit procedures. The day study drug is started is considered Day 0.

2. A clinical echocardiogram within the previous 30 days prior to screening is acceptable for screening purposes.
3. Includes: Sodium, Potassium, Chloride, CO_2
4. Medication dispensing will occur every 90 days, beginning at the time of randomization.

10.3 Day -14 to Day 0 (Screening Evaluations and First Dose)

Informed consent will be obtained prior to the collection of any data and any study-related procedures. Screening evaluations will be obtained. Subjects will undergo a physical, skin, and cushingoid examination, vitals and a review of medical and concomitant medication histories. Echocardiogram, strength testing/dynamometry, and functional testing (100 meter timed test [100M], North Star Ambulatory Assessment [NSAA], 4-stair climb, and time to arise from floor test) will be conducted.

Lab work will include the following:

- Creatine Kinase
- Gamma-glutamyl transferase (GGT)
- Creatinine
- Blood Urea Nitrogen (BUN)
- Glucose
- Serum Cystatin C
- Electrolytes
- Biomarkers blood draw (for plasma and white blood cells)

Once eligibility has been confirmed (including lab results and echocardiogram review), the pharmacy will dispense a 90-day supply of study medication to the research team who will then provide the study medication to the subject's parent/guardian. If eligibility cannot be confirmed on the same day of screening, the study drug may be shipped to the subject and a pharmacist will counsel the parent or guardian via phone. The day study drug treatment is initiated is considered Day 0.

10.4 Week 1 and Month 1 and 2

Subjects will be contacted by research staff via a REDCap survey to review concomitant medications and assess adverse events.

Lab work will include the following:

- Electrolytes

10.5 Month 3

Subjects will return on Month 3 and undergo a physical, skin, and cushingoid examination, vitals. Strength testing/dynamometry, and functional testing (to include 100 meter timed test, North Star Ambulatory Assessment [NSAA], 4-stair climb, and time to arise from floor test) will

be conducted. Research staff will also review concomitant medications, updates to the medical history, and assess adverse events.

Lab work will include the following:

- Gamma-glutamyl transferase (GGT)
- Creatinine
- Blood Urea Nitrogen (BUN)
- Glucose
- Cystatin C
- Electrolytes

The pharmacy will dispense the supply of study medication to the research team who will then provide the study medication to the subject's parent/guardian. If lab results return after the subject leaves the clinic and the Study Doctor wants additional tests, they will be immediately contacted.

10.6 Month 4 and 5

Subjects will be contacted by research staff via a REDCap survey to review concomitant medications and assess adverse events.

Lab work will include the following:

- Electrolytes

10.7 Month 6 (Final Visit)

Subjects will undergo a physical, skin, and cushingoid examination, vitals and a review of medical and concomitant medication histories. Echocardiogram, strength testing/dynamometry, and functional testing (to include 100 meter timed test [100M], North Star Ambulatory Assessment [NSAA], 4-stair climb and time to arise from floor test) will be conducted.

Lab work will include the following:

- Creatine Kinase
- Gamma-glutamyl transferase (GGT)
- Creatinine
- Blood Urea Nitrogen (BUN)
- Glucose
- Cystatin C
- Electrolytes
- Biomarkers blood draw (for plasma and white blood cells)

Research staff will review concomitant medications, updates to the medical history, and assess adverse events.

10.7.1 Long Term Monitoring

All adverse events occurring throughout the subject's participation in the trial through 30 days after the subject's final study visit will be documented and followed until resolution or stabilization. Research staff will contact subjects 30 days after the subject's final study visit to check for any new AEs and to obtain any new information regarding ongoing AEs.

10.8 Concomitant Medications

Prescribed and over the counter medications used in the 2 weeks prior to trial initiation will be recorded at the screening visit and changes in these medications will be recorded during each subsequent medical history review. The Principal Investigator will encourage subjects to maintain the medication and supplements they are on at enrollment through the course of the trial.

10.8.1 Prohibited Medications

Concomitant use of any of the following medications is prohibited:

- ACEi
- Potassium supplementation

11. ASSESSMENT OF SAFETY

One of the primary objectives for this pilot clinical trial is safety. Withdrawal criteria are based on the development of unacceptable toxicity, as described in section 8.5.

11.1 Adverse and Serious Adverse Events

11.1.1 Definition of Adverse Events

All Adverse Events (AE) will be reported to the Reviewing IRB at annual continuing reviews.

AEs will be collected throughout the trial from informed consent to 30 days after the subject's final study visit. All AEs that occur after any subject has been enrolled, before treatment, during treatment, or within 30 days after the last study visit, whether or not they are related to the study, must be recorded in the eCRF.

Adverse Event: Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Adverse events will be graded by the investigator and as used in the CTCAE v.4.03 accordingly:

1. Mild
2. Moderate
3. Severe
4. Life threatening or debilitating
5. Fatal.

Adverse Reaction: An adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

Suspected Adverse Reaction (21 CFR 312.32(a)): A suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. 'Reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug. Suspected adverse events will be reported to the Reviewing IRB at study closeout.

11.1.2 Unexpected Adverse Events (UAE)

Unexpected adverse events are those which are not previously reported with the subject's underlying disease or with the procedures to be used in this trial, or are related to a known toxicity but differ because of greater severity or specificity.

11.1.3 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the Sponsor-Investigator or Principal Investigator, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. **A serious adverse events refers to a grade of 3, 4, or 5.**

All SAEs that occur after any subject has signed informed consent, before treatment, during treatment or within 30 days following the last study visit, whether or not they are related to the trial must be recorded.

11.1.4 Life-threatening (21 CFR 312.32(a))

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the Sponsor-Investigator or Principal Investigator, its occurrence places the subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

11.2 Relationship to Study Drug

Association or relatedness to the study agent, study procedures and the subject's pre-existing disease will be graded as follows:

1. Definitely related
2. Probably related
3. Possibly related
4. Unlikely related
5. Unrelated

If the relationship between the AE/SAE and the investigational product is determined to be "possible" or "probable", the event will be considered to be related to the investigational product for the purposes of expedited regulatory reporting.

11.3 Time Period and Frequency for Event Assessment and Follow-Up

11.3.1 Obligations of the Principal Investigator

All serious adverse events should be reported to the Sponsor-Investigator regardless of severity, relatedness, or expectedness. The Principal Investigator will provide an electronic report to the Sponsor-Investigator who will report to the Reviewing IRB on any serious adverse event that is both unexpected and related to the use of the corticosteroid product (i.e. there is reasonable possibility that the event may have been caused by the use of the product; the Principal Investigator will not await definitive proof of association before reporting such events).

The Principal Investigator will adhere to any other serious adverse event reporting requirements in accordance with federal regulations, state laws, and the local institutional policies and procedures, as applicable.

The Principal Investigator will be responsible for ensuring that the reporting requirements are fulfilled and will be held accountable for any reporting lapses.

11.3.2 Safety Reporting

All serious adverse events that are **unexpected** and **possibly**, **probably**, or **definitely related** to the use of either study drug should be reported to the Sponsor-Investigator within **48 hours** of the Principal Investigator's initial receipt of the information. All other serious adverse events

should be reported to the Sponsor-Investigator within **7 days** of the Principal Investigator's initial receipt of the information.

The Sponsor-Investigator will report serious adverse events that are unexpected and possibly, probably, or definitely related to the use of either study drug to the Reviewing IRB according to regulatory requirements described as follows:

Any serious adverse event that is fatal or life-threatening, that is unexpected and related to the use of either study drug will be reported to the Reviewing IRB as soon as possible, but not later than **3 business days** after the Sponsor-Investigator's initial receipt of the information.

Serious adverse events that are unexpected and related to the use of either study drug, but are not fatal or life-threatening, will be reported to the Reviewing IRB as soon as possible, but not later than **3 business days** after the Sponsor-Investigator's initial receipt of the information.

If, after further evaluation, an unexpected serious adverse event initially considered not to be related to the use of either study drug is subsequently determined to be related, then the event will be reported to the Sponsor-Investigator within **48 hours** of the determination. The Sponsor-Investigator will report to the Reviewing IRB within **3 business days** of the Sponsor-Investigator learning of the determination.

Relevant additional clinical and laboratory data will become available following the initial serious adverse event report. Principal Investigators must report follow-up information to the Sponsor-Investigator within **48 hours** of initial receipt of the information. All follow-up information relevant to a serious adverse event that is unexpected and related to the use of either study drug will be reported to the Reviewing IRB within **3 business days** of the Sponsor-Investigator's initial receipt of the information.

If a serious adverse event occurs within 30 days after a subject's final study visit and is determined to be unexpected and related to the use of either study drug, that event will be reported to the Reviewing IRB within **3 business days** of the Sponsor-Investigator learning of the determination.

Should a serious adverse event deemed possibly, probably or definitely related to the study agent occur during administration, the study agent will be discontinued, appropriate treatment will be given under medical supervision and the subject will be examined as frequently as necessary thereafter until symptoms cease or stabilize.

All new information and significant noncompliance indicating a new or increased risk or new safety issue as defined in the Reviewing IRB policies will be reported to the Sponsor-

Investigator within **48 hours** of the Principal Investigator learning of the information. The Sponsor-Investigator will report to the Reviewing IRB within **3 business days** of the Sponsor-Investigator's initial receipt of the information. This includes deviations from the protocol that harmed subjects or others or that indicates subjects or others might be at an increased risk of harm.

The Sponsor-Investigator will inform the Clinical Safety Monitor of all serious adverse events regardless of severity, relatedness, or expectedness and of all instances of significant non-compliance impacting patient safety within **48 hours** of the Sponsor-Investigator's initial receipt of the information.

11.3.3 Serious Adverse Event Reporting: Content and Format

The serious adverse event report will include, but need not be limited to: (1) the date of the event; (2) designation of the report as an initial report or a follow-up report, identification of all safety reports previously filed for the clinical protocol concerning a similar adverse event, and an analysis of the significance of the adverse event in light of previous similar reports; (3) clinical site; (4) the Principal Investigator; (5) study drug; (6) route of administration e.g. intravenous or oral; (7) dosing schedule; (8) a complete description of the event; (9) relevant clinical observations; (10) relevant clinical history; (11) relevant tests that were or are planned to be conducted; (12) date of any treatment of the event; and (13) the suspected cause of the event. These items will be reported electronically to the Sponsor-Investigator who will then report to the Reviewing IRB as appropriate.

11.3.4 Communication Plan with the Primary Care Physician

Close communication should be established with the primary care physician of all study subjects and will be maintained throughout the trial if necessary. The important hallmarks of the trial will be explained. We may request laboratory reports, hospitalizations, clinical notes and any other relevant medical records pertaining to intercurrent events taking place during this 6-month study. The Principal Investigator will initiate an investigation to determine the possibility of an adverse event related to this trial and will adhere to the adverse event reporting requirements in accordance with federal regulations, state laws, and the local institutional policies and procedures, as applicable.

During the consent process, the Principal Investigator or designated Co-Investigator will emphasize the importance of subject communication with our study team. Any routine or non-routine doctor's visits or medical care received during the 6 months of this trial should be reported to the study team. The Study Doctor will explain to the subject that copies of any relevant medical records of those visits will be requested from their medical care provider.

11.3.5 Follow-Up of Adverse Events

All adverse events will be followed until resolution or stabilization.

11.4 Study Discontinuation Rules

An independent Clinical Safety Monitor will monitor safety data on a continual basis throughout the trial. The Clinical Safety Monitor can recommend early termination of the trial for reasons of safety. Study enrollment will be halted by the Principal Investigators when any subject experiences a Grade 3 or higher adverse event toxicity that is unanticipated and possibly, probably, or definitely related to the study drug that presents with clinical symptoms and requires medical treatment. This will include any subject death, hospitalization, or important clinical laboratory finding. If after review by the Clinical Safety Monitor and the Reviewing IRB, the decision is made to continue, the trial will proceed according to Section 10 of this protocol.

Anticipated adverse events related to progression of Duchenne muscular dystrophy may include muscle weakness, elevated creatine kinase levels, loss of ambulation, scoliosis, short stature, and intellectual disability.

Anticipated adverse events related to prednisolone treatment, which is the standard of care, may include weight gain, cushingnoid features, long bone fractures, acne, behavioral disturbance, and short stature (see section 9.3.2 for additional potential side effects).

Anticipated adverse events related to spironolactone treatment may include increased potassium levels, increased breast tissue mass, diarrhea, cramping, nausea, vomiting, headache, sleepiness, unsteadiness, and urticaria (see section 9.3.1 for additional potential side effects).

12. CLINICAL MONITORING

12.1 Data Safety Monitoring Plan

Dr. Richard Shell will serve as Clinical Safety Monitor for this trial. Dr. Shell is a pediatric pulmonologist and critical care specialist at NCH, and an Associate Professor of Pediatrics at the Ohio State University. Dr. Shell will be asked to review all subjects' laboratory results monthly. He will be apprised of all adverse events, as per the Reviewing IRB policy. He will prepare interim safety reports 3 months and 6 months after the first subject is dosed and a final safety report after all subjects complete 6 months of dosing. The Clinical Safety Monitor can recommend early termination of the trial for reasons of safety.

The Clinical Safety Monitor's responsibilities are to:

- review the plans for data and safety monitoring;

- consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on participant safety or the ethics of the trial;
- review study performance, make recommendations and assist in the resolution of problems reported by the Principal Investigators;
- review monthly safety data to determine safety signals or trends;
- ensure the confidentiality of the trial data; and,
- assist by commenting on any problems with study conduct, enrollment, and sample size and/or data collection.

12.1.1 Safety Monitor Reporting

Reports describing the status of the study will be prepared by the Sponsor-Investigator and associated staff and sent to the Clinical Safety Monitor the end of each month, or at the Clinical Safety Monitor's request.

Reports will include the following:

- A brief narrative of the trial status, including the target enrollment, current and projected time to completing enrollment. Any significant events and/or difficulties should be briefly described in this narrative;
- A brief narrative for each subject describing age, race and ethnicity and other relevant demographic characteristics. The narrative for each subject should briefly describe his study status (i.e., dose level, visit number, adverse event information);
- A timeline outlining the trial progress relative to visit number for each subject, as well as time points for each SAE. A total for Adverse Events (AEs) for each subject should be included;
- A summary of AEs by classification;
- A listing of AE details grouped by subject;
- A listing of SAE details grouped by subject;
- A listing of deaths;
- A summary of clinically significant laboratory test results;

12.2 Institutional Review Board Monitoring & IRB Reliance

The Principal Investigators will obtain IRB approval for the investigation. Initial IRB approval, and all materials approved by the Reviewing IRB for this trial, including the subject's consent forms and recruitment materials, must be maintained by the Principal Investigators and made available for inspection.

The NCH IRB has offered to serve as the Reviewing IRB for all institutions via SMART IRB. Participating sites of this protocol are encouraged to utilize this infrastructure to expedite the review process. Participating sites will need to be existing members of the SMART IRB Master

Common Reciprocal Institutional Review Board Authorization Agreement to utilize the NCH IRB as their IRB of record. Sites that use the single IRB will rely on the NCH IRB approval in order for all study sites to have the same IRB determination, consent template(s), and expiration date.

Sites that choose not to use the NCH IRB as IRB of record must provide the Coordinating Center with a copy of the initial IRB protocol approval and the yearly IRB continuing reviews.

Modifications will be submitted to the NCH IRB for their review and approval prior to implementation. When a modification to a protocol substantially alters the study design or increases potential risk to the study subject, the informed consent form will be revised and if applicable, subject's consent to continue participation will again be obtained.

13. STATISTICAL CONSIDERATIONS

Age-corrected 100M will be considered as the primary outcome, and will be evaluated using a two-one-sided t-test to test equivalency between treatment arms in the change from baseline to 6 months. In addition, repeated measures ANOVA will be used to determine whether the trajectory across all three time points (baseline, 3mo, 6mo) differs by treatment group. Strength testing/dynamometry, NSAA, 4-stair climb, and time to arise from floor scores will be secondary outcomes, and will also be compared by treatment group using repeated measures ANOVA. Biomarkers studies will be considered exploratory outcomes.

Power: Prior studies evaluating the 100m walk test among patients with DMD have shown an average change over 6 months of -2.9 ±7.8. An effective sample size of 10 patients per group will provide 80% power to declare equivalency with an equivalence margin of ±10.6[21].

14. SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

14.1 Data Management and Study Forms

Research material obtained from identifiable living human subjects includes reports of: history and physical assessments, blood, strength testing/dynamometry testing, NSAA, 4-stair climb, time to arise from floor, clinical assessments, adverse events, and autopsies. Copies of case report forms, original test results, subject medical records, signed subject informed consents, correspondence, and any other documents of the subjects, relevant to the conduct of the trial will be kept on file by the Principal Investigator. All material or data collected as part of the trial will be obtained specifically for research purposes.

In collaboration with the study team, the Research Informatics Core designed a data collection system (Open Clinica) for managing the clinical trial. A web-based database was created and it will be managed by authorized users. CRFs will be transcribed to this web-based database via eCRFs. Data will be extracted from source documents (lab reports, echo reports, etc.) and transferred to the database as well. All source documents will be kept in the subject research binder. The secured portal will feature view and edit capability with field validations for quality controls, change history attribute, and reporting.

An additional data collection system (REDCap) has been developed to help assess adverse events and concomitant medications remotely. REDCap surveys will be sent by each site to subjects' parent(s)/guardian at week 1 and months 1, 2, 4 and 5. These timepoints require blood testing for electrolytes which can be done offsite, without a visit with the study team. Responses to the surveys will be reviewed to ensure all adverse events and concomitant medications are recorded. Follow-up phone calls will be done when necessary.

14.2 Electronic Case Report Forms (eCRF)

Adequate and accurate case records will be maintained and all relevant observations and data related to the trial will be recorded. This will include medical history/physical examination, hematology, clinical chemistry and serology results, etc. as specified in this protocol. All data and observations will be documented on electronic Case Report Forms (eCRF) by source documentation using the Open Clinica Electronic Data Capture designed for the trial.

A study monitor from NCH will have access to the data to monitor adherence to the protocol and to applicable FDA regulations, and the maintenance of adequate and accurate clinical records. An electronic Case Report Form (eCRF) will be completed for every subject that was registered for participation in the trial. The eCRFs will be completed as information becomes available.

Completed eCRFs will be reviewed by the study monitor in detail on a regular basis against the source documentation for accuracy and completeness. The monitor will make a decision as to the data acceptability. If errors or omissions are found in the course of a data audit, or if clarification of data is required, the eCRF(s) in question will be corrected by the PI or the PI's designee. Data resolution may be generated on omissions or clarifications, to be completed, electronically signed and dated, and maintained as a part of the eCRF.

The Principal Investigator or the designated Co-Investigator will sign and accept the indicated eCRF. This signature will indicate that thorough inspection of the data therein has been made and will thereby certify the contents of the form. After the completion of the trial, completed eCRFs will be retained in the archives.

14.3 Inspection of Records

The Principal Investigator agrees to allow the study monitor to inspect the drug storage area, study drug stocks, drug accountability records, subject charts, study source documents, and other records relative to study conduct.

15. QUALITY ASSURANCE AND QUALITY CONTROL

15.1 Study Monitoring

NCH will act as the central monitor for the study. The trial will be monitored in compliance with the relevant parts of 21 CFR and according to the ICH GCP Guidelines. During the trial, the study monitor will have regular contact with the investigational site to provide information and support to the Principal Investigator(s) and to confirm that the investigational team is adhering to the protocol and ICH/GCP guidelines.

No deviations from the protocol shall be made except in emergency situations where alternative treatment is necessary for the protection, proper care and well-being of subjects. Protocol deviations will be monitored throughout the study.

The procedures outlined in the protocol and case report forms will be carefully reviewed by the PI and staff prior to study initiation to ensure appropriate interpretation and implementation. Copies of all documentation essential for site initiation (e.g., notification of national/local regulatory and ethical approval and any other permissions required, copies of CVs for all site staff, completed delegation and signature log, etc.) will be reviewed by the study monitor prior to site authorization/initiation.

The study monitor will review new and/or updated site study data (as available) and call each site monthly, beginning 30 days after enrollment of the first subject, and at close out. Monthly calls can be forgone if no subject visits have occurred in the previous month. The study monitor will be available between visits if the Principal Investigator(s) or other staff needs information or advice.

The study monitor will perform the following monthly as appropriate and available:

- Review available on-site resources (personnel, space, equipment, milieu);
- Discuss the study protocol and visit schedule with staff to ensure familiarity;
- Review maintenance of study records;
- Review the informed consent forms executed by participating research subjects, and the process by which consent was obtained;
- Review submitted study data to ensure consistency with source documentation, and to ensure study data have been collected in an accurate, complete and timely manner, consistent with the protocol and GCP's;

- Review REDCap responses for subjects enrolled at the site and compare the responses to study data to ensure adequate reporting of adverse events and recording of concomitant medications;
- Review site IRB approvals and regulatory documentation (e.g. Clinical Trial Authorizations) to ensure all such regulatory and ethical documents are correct and up to date;
- Assist the sites with resolution of electronic data clarifications (queries);
- Ensure that all serious adverse events have been properly and promptly reported;
- Ensure that laboratory results conform to study standards

16. ETHICS/PROTECTION OF HUMAN SUBJECTS

16.1 Ethics Review

The final study protocol, including the final version of the informed consent forms, must be approved or given a favorable opinion in writing by the Reviewing IRB. The Sponsor-Investigator is responsible for informing the Reviewing IRB of any amendment to the protocol in accordance with the Reviewing IRB's requirements. The protocol must be re-approved by the Reviewing IRB upon receipt of amendments and annually, as required by Nationwide Children's Hospital.

16.2 Ethical Conduct of the Trial

The trial will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, and applicable regulatory requirements.

16.3 Written Informed Consent

Legally effective and properly executed written informed consent, in compliance with 21 CFR 50 and the International Conference on Harmonization (ICH) guidelines, will be obtained from each subject before the subject is entered into the trial or before any unusual or non-routine procedure is performed that involves risk to the subject. At subject enrollment, the Principal Investigator or designated Co-Investigator will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the trial. Subjects must also be notified that they are free to discontinue from the trial at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

Attention will be directed to the basic elements that are required for incorporation into the informed consent under US Federal Regulations for Protection of Human Subjects [21CFR 50.25(a)]. If new information related to the trial arises, subjects may be asked to sign a revised document depending on the changes. Initial consent will be obtained only by the Principal

Investigator or a designated Co-Investigator delegated the task of initial consenting. For any changes requiring re-consent of the subject, re-consent can be performed according to the procedures defined in this section of the protocol by a study team member delegated the task of re-consenting by the Principal Investigator. Signed consent forms will remain in each subject's research chart and be available for the verification by study monitors at any time. The Principal Investigator must maintain the original, signed ICF. Subjects will be given a signed, dated copy of their consent form documents.

Signatures are required from both parent(s)/legal guardian(s) of the subject in order to consent for the subject's participation in the study due to the risk level of the study as determined by the NCH IRB. All parent(s)/guardian(s) must be present to consent in person for initial consent. Prior approval from the Reviewing IRB must be obtained if extreme circumstances will only allow for one parent/guardian to be present in person and the other present by telephone for initial consent. For changes requiring re-consent, re-consent from both parent(s)/guardian(s) can be obtained at the subject's next in-person visit unless the changes warrant immediate re-consent between visits. No prior approval from the Revieweing IRB is necessary to obtain re-consent from one or both parent(s)/guardian(s) via phone unless otherwise required by local site policies.

17. DATA HANDLING AND RECORD KEEPING

17.1 Retention of Records

All primary data that are a result of the original observations and activities of the trial and that are necessary for the reconstruction and evaluation of any study report will be retained in a secure archive at the study site for a period of at least 2 years after the formal closure of the study. These documents should be retained for a longer period, however, if required by the applicable regulatory requirement(s) or if needed by the Sponsor. The Sponsor should inform the investigator(s)/institution(s) in writing of the need for record retention and should notify the investigator(s)/institution(s) in writing when the trial-related records are no longer needed.

If the investigator is no longer able to fulfill their record retention obligations (for example if they leave to work at another institution, or retires), they should make arrangements of who will ensure that the documents are retained securely while maintaining subject confidentiality.

The site will maintain a research regulatory binder. In this binder, there will be tabbed sections for study documents including, but not limited to, the following: study personnel identification and signature list, subject screening records, subject roster (names omitted), protocol and amendments or administrative changes, FDA Form 1572 (if required), study staff Curricula Vitae, Reviewing IRB documentation, an approved sample ICF, drug accountability records, correspondence, site monitoring reports, and lab accreditations and normal values. The site must

keep this binder current and available for review by the Sponsor, Reviewing IRB, FDA, and other similar regulatory bodies.

17.2 Retention of Samples

The identified storage laboratory will be responsible for arranging storage of any remaining or unused biological samples as well as properly documenting the storage procedures, once all study-required analyses are complete, as per sample processing requirements until such time that the Sponsor-Investigator provides external storage vendor transfer or destruction instructions.

17.3 Study Reports

17.3.1 Annual Study Reports

Annual continuing reviews will be submitted to the Reviewing IRB, including information about current study status, study amendments, changes in study risk and adverse event reporting, targeted and completed study enrollment, subject withdrawal, summary of study findings, or publication information.

17.3.2 Final Study Report

The final study report will include data through the final study visit.

18. STUDY ADMINISTRATION

18.1 Coordinating Center

The Nationwide Children's Center for Gene Therapy at the Abigail Wexner Research Institute is the Coordinating Center for the entire trial.

The Coordinating Center is responsible for the following:

- Development and maintenance of the Manual of Operating Procedures
- Training and reliability testing of additional sites
- Enrollment of subjects at NCH
- Curating data in the database
- Obtaining informed consent from subjects at NCH
- Adverse event monitoring and reporting

18.2 Overview of Principal Investigator Responsibilities

In conducting the clinical trial, each site's Principal Investigator is responsible:

- For ensuring that a clinical investigation is conducted according to applicable regulations;
- For protecting the rights, safety, and welfare of subjects under the investigator's care;
- For the control of the study drug under investigation;

- To conduct the trial in accordance with the relevant, current protocol(s) and to only make changes in the protocol after notifying the Sponsor-Investigator, except when necessary to protect the safety, rights, or welfare of subjects;
- To personally conduct or supervise the research study;
- To inform the subjects, that the drugs are being used for investigational purposes and to ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met;
- To report to the Sponsor-Investigator adverse experiences that occur in the course of the investigations(s) in accordance with 21 CFR 3 12.64;
- To ensure that all associates, colleagues, and employees assisting in the conduct of the trial are informed about their obligations in meeting the above commitments;
- To maintain adequate and accurate records and to make those records available to the FDA and other similar regulatory bodies for inspection in accordance with 21 CFR 3 12.68;
- That Reviewing IRB will be responsible for the initial and continuing review and approval of the clinical investigation;
- To promptly report directly to the Sponsor-Investigator for appropriate reporting to the Reviewing IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others;
- To not make any changes in the research without the Reviewing IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects;
- To comply with all other requirements regarding the obligations of Principal Investigators and all other pertinent requirements in 2 1 CFR 3 12.

19. CONFLICT OF INTEREST POLICY

The Principal Investigators and Co-Investigators will have no financial, scientific or other conflicts of interest with the trial. Conflicts of interest can include professional, proprietary, and miscellaneous interests as described in the NIH Grant Policy Statement and 45 CFR Part 94.

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